

1 Supplemental Information for
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4 **An alpaca nanobody neutralizes SARS-CoV-2 by blocking receptor interaction**
5 by
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12 **Supplemental Table 1 Cryo-EM data collection, refinement and validation statistics**

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| | #1 Spike-Ty1 (EMDB-11526) (PDB 6ZXN) |
| Data collection and processing | |
| Magnification | 165,000x |
| Voltage (kV) | 300 kV |
| Electron exposure (e-/Å ²) | 38 |
| Defocus range (μm). | 0.5 – 2.0 microns |
| Pixel size (Å). | 0.51 Å |
| Symmetry imposed | C1 |
| Initial particle images (no.) | 573,036 |
| Final particle images (no.) | 210,832 |
| Map resolution (Å) FSC threshold | 0.143 FSC |
| Map resolution range (Å) | 2.9 Å |
| | |
| Refinement | |
| Initial model used (PDB code) | 6VSB, 5JMR, 6LZG |
| Model resolution (Å) FSC threshold | 2.9 Å 0.143 |
| Model resolution range (Å) | 2.9 – 3.6 |
| Map sharpening <i>B</i> factor (Å ²) | -67 |
| Model composition | |
| Non-hydrogen atoms | 26297 |
| Protein residues | 3261 |
| Ligands | 67 (NAG) |
| <i>B</i> factors (Å ²) | |
| Protein | 137 |
| Ligand | 129 |
| R.m.s. deviations | |
| Bond lengths (Å) | 0.012 |
| Bond angles (°) | 1.459 |
| Validation | |
| MolProbity score | 2.87 |
| Clashscore | 38.66 |
| Poor rotamers (%) | 0.10 |
| Ramachandran plot | |
| Favored (%) | 95.53 |
| Allowed (%) | 4.47 |
| Disallowed (%) | 0.00 |

14 **Supplemental Table 2 List of primers used in the study.**

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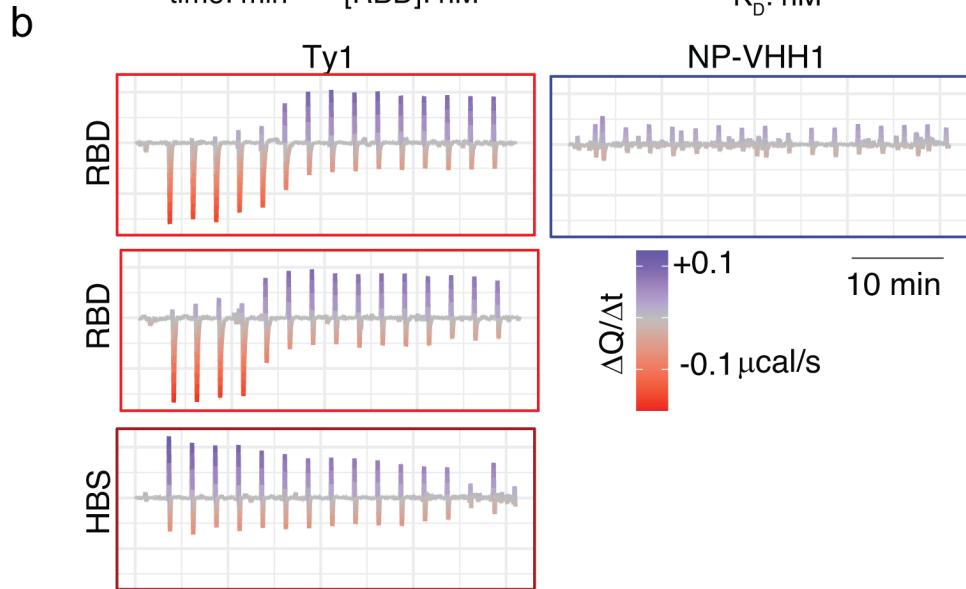
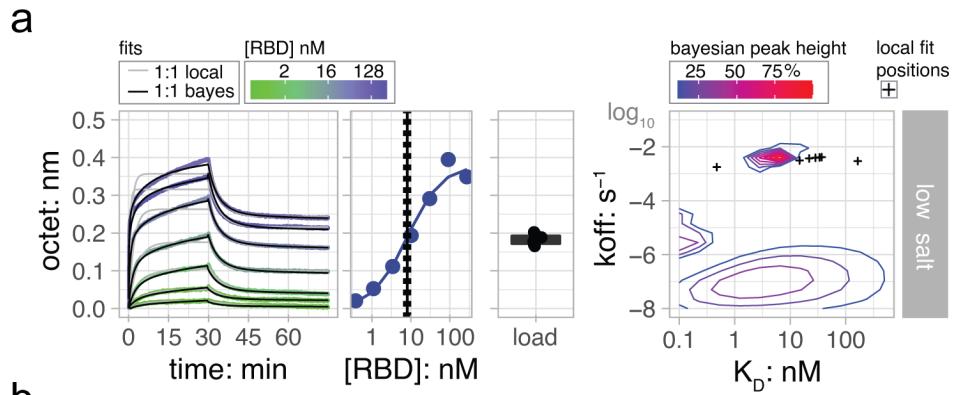
| Description | Name | Sequences |
|--|------------|--|
| Gene specific primers for cDNA synthesis | AL.CH2 | ATGGAGAGGACGTCCCTGGGT |
| | AL.CH2.2 | TTCGGGGGAAAGAYRAAGAC |
| Primers for library amplification and cloning into phagemid plasmid | AIVHH-F1 | CTTGCAGGCCGCTCAGKTGCAGCTCGTGGAGWCNGGNNG |
| | AIVHH-shR1 | GATCGGCGCGCCGAGGGTCTCGCTGTGGTGCG |
| | AIVHH-lhR1 | GATCGGCGCGCCGGTTGTGGTTTGGTGTCTGGG |
| Next generation sequencing primers | NB-NGS-FW | CACTTTCCCTACACGACGCTTCCGATCTCTCGCGGCC CAGCCGGCCATGG |
| | NB-NGS-RV | GGAGTTCAGACGTGTGCTTCCGATCTACCGGCGCACCA CTAGTGCA |
| Primers for cloning nanobodies into pHEN plasmid using Gibson assembly | nb-FW | CGCGGCCAGCCGCCATGGCCCAGGTGCAGCTCGTGG |
| | nb-con-Rev | AGTCCTCCTGAGGAGACGGTGACCTGGTCCCCTGGCC |

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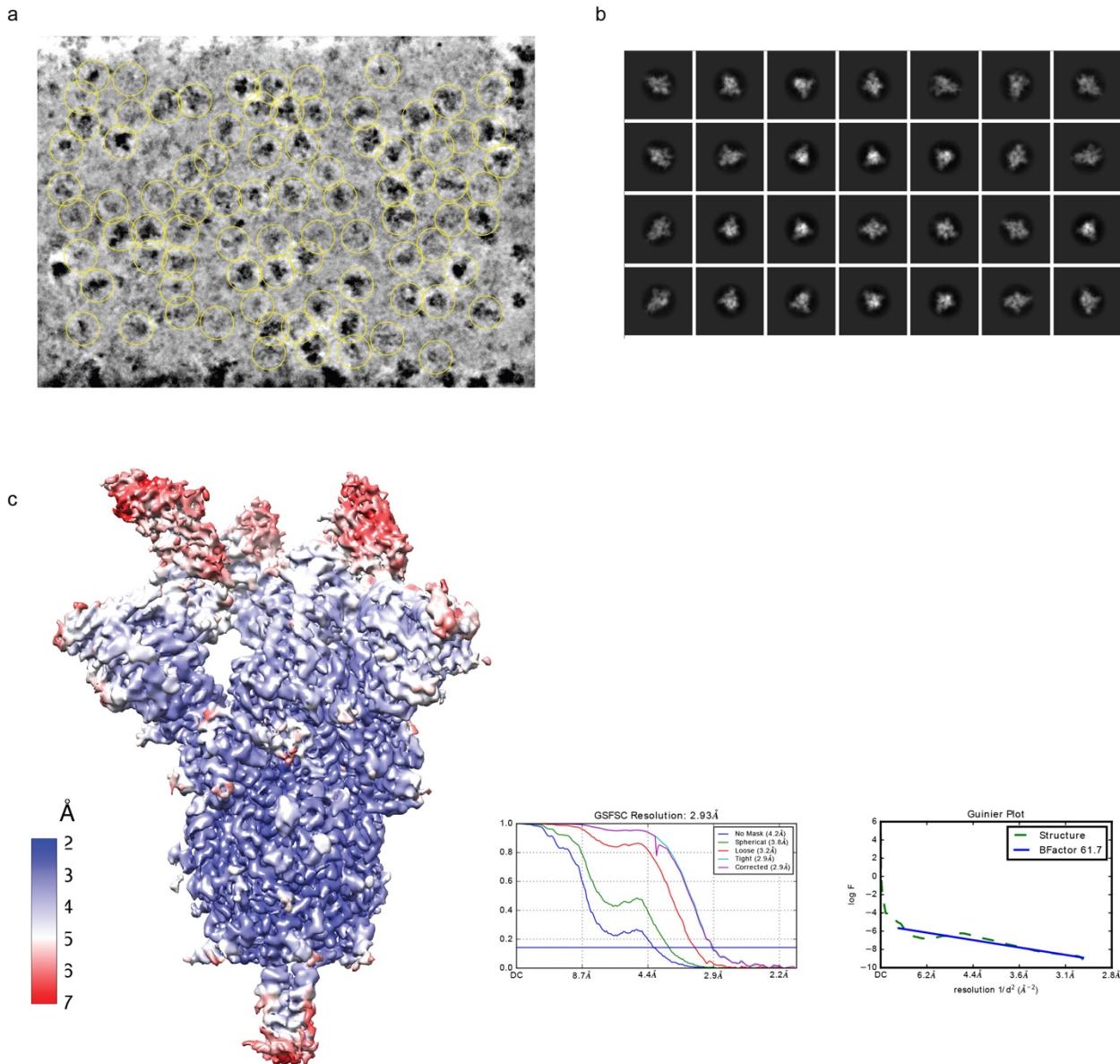
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Supplemental Figure 1 a, related to Fig 3b, plots for RBD/Ty1 titrations at low salt condition with same legends and scales. **b**, related to Fig 3c, Same data plotted on larger scale to highlight the Ty1 into buffer dilution spikes.



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28 Supplemental Figure 2 Cryo-EM processing

29 a, Representative micrographs. For clarity, the micrographs has been denoised using Warp.
 30 Particle autopicked by Warp are denoted with a yellow ring with 200 Å diameter in the sample
 31 plane. b, Representative 2D classes from CryoSPARC. c, Local reconstruction resolution
 32 mapped to a locally sharpened map. d, FSC curve and Guinier plot as calculated by
 33 CryoSPARC.

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